

Recruitment in a freshwater unionid (Mollusca: Bivalvia) community downstream of Cave Run Lake in the Licking River, Kentucky

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Abstract: Unionids, fish, and glochidia were collected to determine why recruitment had ceased or had been dramatically decreased in a speciose unionid community in the Licking River at Moores Ferry, Kentucky, 35.4 km downstream of Cave Run Lake. Only six unionid glochidia were collected with drift nets, and only six fish collected had infestations of glochidia. A small percentage (10.1%) of the unionids observed had their gills modified as marsupia. An analysis of water temperature and discharge indicated no significant difference in average monthly discharge ($p > 0.05$) and a significant decrease in temperature ($p < 0.05$) between pre- and post-impoundment periods. Average monthly discharge and temperature may not be as biologically important as the spikes of discharge and corresponding sudden decreases in temperature that are caused by releases of hypolimnionic water from the reservoir.

Key Words: Unionidae, recruitment, gametogenesis, impoundments, Kentucky

North America has the richest unionoidean (mussel) (Bivalvia: Unionidae) fauna in the world. This fauna has disproportionately more endangered, threatened, and special concern taxa than all the groups of terrestrial organisms. Only 70 of the 297 taxa known from the United States are considered stable (Williams *et al.*, 1993). Of the freshwater unionid taxa recognized from the United States and Canada, 35% (103 taxa) are known to occur, or have occurred in Kentucky, which ranks this state third in faunal richness behind Tennessee and Alabama (Cicerello *et al.*, 1991). Human activities in the Commonwealth have severely impacted unionid populations during the last 200 years, making this group of organisms the most endangered in the state (Cicerello *et al.*, 1991).

Freshwater mussels are impacted by anthropogenic factors such as the additions of toxic substances into aquatic systems, sedimentation, habitat destruction, loss of their host fish(es), introduced species, and commercial harvesting (van der Schalie and van der Schalie, 1950; Fuller, 1980; Bogan, 1993; Williams *et al.*, 1993). The construction of dams along the course of several rivers in North America has resulted not only in the loss of taxa, but also of whole beds (*e. g.*, van der Schalie and van der Schalie, 1950; Bates, 1962; Miller *et al.*, 1984; Miller *et al.*, 1992; Williams *et al.*, 1992; Layzer *et al.*, 1993; Sickel and

Chandler, 1996). One of the most severe and perplexing problems facing freshwater mussels is the documented loss of recent recruitment (reproduction) in unionid communities that were previously thought to be healthy.

Recent research hypothesized that recruitment had ceased or had been dramatically decreased in a diverse unionid community in the Licking River at Moores Ferry, Kentucky (Kane, 1990; Smathers, 1990). The present study was an attempt to determine if recruitment had ceased or had been decreased in this diverse unionid community, and if so, to determine where in the life cycle reproduction was breaking down. This study was also an attempt to determine what effects, if any, the hypolimnionic discharges from Cave Run Lake were having on the mussels in the bed at Moores Ferry. It was hypothesized that the loss of recruitment in this bed was either directly or indirectly the result of the dam, located approximately 35.4 km upstream, which has altered both the natural temperature and flow regimes of the river.

METHODOLOGY

STUDY AREA

The Licking River is a sixth order tributary to the Ohio River, originating on the Unglaciaded Allegheny Plateau in the Appalachian Province of eastern Kentucky. The river flows through an extremely variable topography in a northwesterly direction through the Blue Grass region

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of the Commonwealth for 496 km until its confluence with the Ohio River near Covington, Kentucky, at Ohio River km 757.2 (Harker *et al.*, 1979, Hannan, *et al.*, 1982, Burr and Warren, 1986). This drainage encompasses approximately 10% of the Commonwealth (9601 km²) and covers all or a portion of 21 counties (Harker *et al.*, 1979).

In 1974 the Licking River was impounded to form Cave Run Lake, a warm oligotrophic lake (Clinger, 1974). The lake has a surface area of 3347 ha that inundates 61 km of the mainstem and the lower reaches of several small tributaries (Burr and Warren, 1986). The Licking River drainage has a speciose unionid fauna with 53 taxa, over half of the state's native mussel fauna, historically existing in the drainage (Cicerello *et al.*, 1991). A recent survey below the reservoir indicated that 50 taxa still reside in that portion of the river (Laudermilk, 1993).

Moore's Ferry, Kentucky, is a ford approximately 35.4 km downstream from Cave Run Lake (Fig. 1). The watershed at this site is utilized mainly for agriculture. Discharge at this site is affected by releases of water from Cave Run Lake, which at times causes drastic water level fluctuations. Substrata consisted mainly of cobble, gravel, rubble, and some boulders with intermixed sandy areas (Smathers, 1990). The unionid community at this site had a rich assemblage of unionid species, with 35 known present or historical taxa (Smathers, 1990; Laudermilk, 1993).

SAMPLING AND LABORATORY PROCEDURES

Five collections of glochidia, fish, and unionids were made from July through October 1995. High water conditions prevented collections during Spring 1996. For each collection period a drift net was haphazardly placed in the bed to collect glochidia. After one hour, the contents of the drift net were removed and preserved in 70% ethanol and returned to the laboratory. Drift net collections were examined using cross-polarized light microscopy (Johnson, 1995), with a portion of the sample being delivered into a watch-glass with a gridded bottom. Each square in the grid was then systematically searched under cross-polarized light (10-20X) for glochidia, which were counted and removed along with any juvenile *Corbicula fluminea* (Müller, 1774).

Fish were collected for one hour using a minnow seine. All fish retained were initially preserved in a 10% formalin solution and then transferred to 70% ethanol in the laboratory for final preservation. Following sorting and identification, the fins and scales of each individual were examined under a dissecting microscope (10-30X) for attached glochidia. The opercular flaps were removed, and each gill arch was carefully examined under a dissecting microscope for attached glochidia (Bruenderman and Neves, 1993).

Unionids were collected by hand for one hour by

snorkeling, or by wading with the use of water scopes. After identification, the shell of each unionid was carefully opened with a small screwdriver, and the gills examined for signs of gravidity. The species name was recorded and notes were made on the condition of the gills. Except for individuals of two target species retained for histological examination, all unionids were returned to the river. Three to five individuals of the two most common species in the bed at Moore's Ferry (Smathers, 1990), *Actinonaias ligamentina* (Lamarck, 1819) and *Elliptio dilatata* (Rafinesque, 1820), were chosen for histological examination from each collecting period. These species represented both of the breeding regimes of freshwater mussels, they were both commonly encountered throughout their respective ranges (Oesch, 1995), and neither had any federal or state protection status in Kentucky (Kentucky State Nature Preserves Commission [KSNPC], 1996).

Individuals were prepared for histological examination by placing them into a 10% formalin solution, then transferring them to 70% ethanol in the laboratory. The valves were opened by cutting the adductor muscles and portions of the gonadal and gill tissues were removed and placed into either 70% ethanol or Bouin's fixative. These were then dehydrated through a series of alcohols and embedded in paraffin (Humason, 1967). Sections were made at a thickness of 10 µm using an American Optical 820 Microtome. Slides were stained with Ehrlich's hematoxylin and eosin was used as the counterstain (Humason, 1967). Sections were mounted with Permount. The sections were then examined under a compound microscope (400-430X) to determine a sex ratio for both species; to determine if gametogenesis was occurring, and, if so, to try to quantify it; and to determine the contents of the marsupia. All drift net, fish, and unionid collections were deposited in the Branley A. Branson Museum of Zoology, Eastern Kentucky University (EKU).

Five cell types of spermatogenesis (Garner, 1993) were used to determine the stage of gametogenesis in males. Stage 1 males were those that had only spermatogonia present in their acini, and Stage 5 males had mature spermatozoa present. Stages 2, 3, and 4 corresponded respectively to sperm morulae, spermatocytes, and spermatids being present in the acini. Three cell types of oogenesis (McMurray, 1997) were used to determine the stage of gametogenesis in female *Elliptio dilatata*. Stage 1 females were those with oogonia as the dominant cell type in their alveoli, Stage 2 were those with oocytes dominant, and Stage 3 were those with mature ova dominant.

Marsupia were classified according to their contents as being empty (EM), or containing mature glochidia (MG), early embryos (EE), or advanced embryos (AE) (Garner, 1993). In the case of known females that did not have their gill tissues examined, the marsupia were consid-

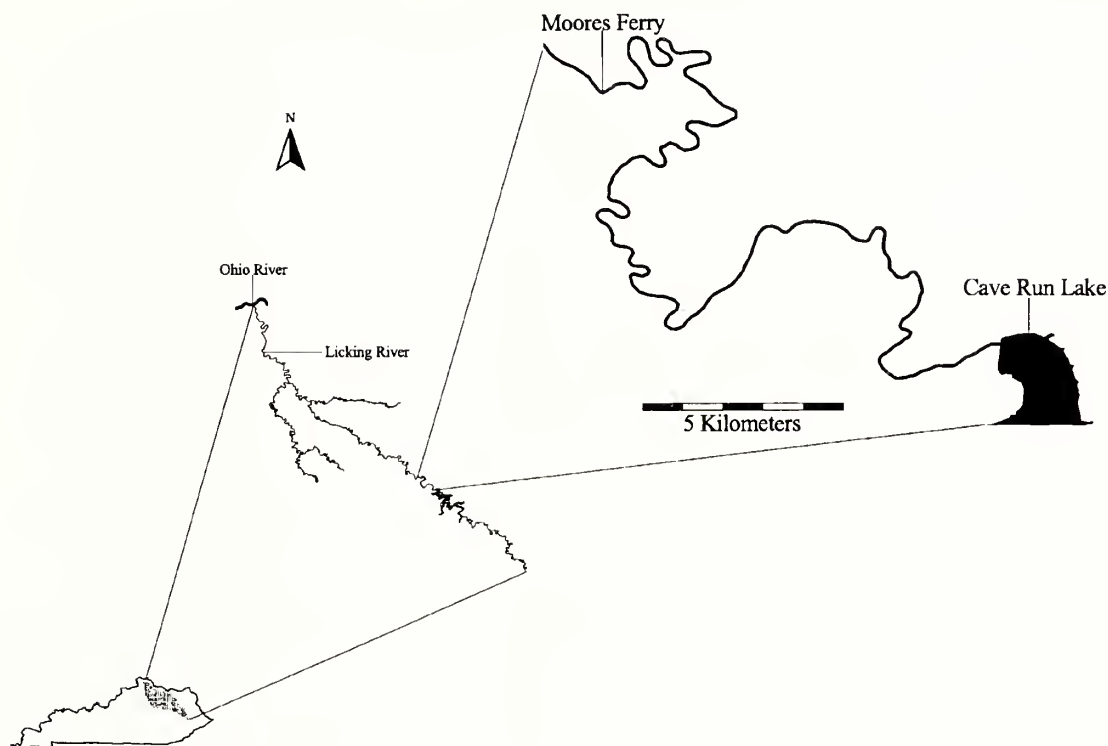


Fig. 1. Location of the Moores Ferry study site. Inset shows the location of the Licking River drainage in Kentucky.

ered to be empty since sections were made of any gill that showed signs of gravidity. Marsupia with mature glochidia were those that had glochidial shells and the single adductor muscle visible in the section.

Temperature and discharge data were obtained from the United States Geological Survey (USGS) for the Licking River gauging station at Farmers, Kentucky, which is just upstream of Moores Ferry. Data were obtained for a pre-impoundment (1966-1970) and a post-impoundment (1991-1994) period. Daily temperature and discharge data from the months of May through September for each of these years, with the exception of 1994 (May-June), were analyzed to determine if any correlation existed between discharge and water temperature.

RESULTS AND DISCUSSION

A total of only six unionid glochidia were collected using drift nets, compared to 185 juvenile *Corbicula fluminea*. The large number of juvenile *C. fluminea* was largely the result of a single collection of 127 individuals. Drift net collections were made between 1000 and 1700 hours (EST), which corresponds to the period when glochidial densities should have been at their highest (Kitchell, 1985). The high number of *C. fluminea* present may impact juvenile unionids through resource competition (Neves and

Widlak, 1987).

Only 11.3% of the 399 fish collected were suitable hosts for unionids in the bed (Watters, 1994). Of the fish collected only six specimens had attached glochidia: one *Micropterus punctulatus* (Rafinesque, 1819); three *Percina copelandi* (Jordan, 1877); one *P. evides* (Jordan and Copeland, 1877); and one *P. oxyrhyncha* (Hubbs and Raney, 1939). Even though a small fish may successfully carry several hundred glochidia (Lefevre and Curtis, 1910), low infestation rates appear to be common (Kitchell, 1985; Neves and Widlak, 1988; Bruenderman and Neves, 1993; Weiss and Layzer, 1995). The attachment of glochidia to their hosts is dependent upon several factors such as infestations of hosts by copepod parasites (Wilson, 1916), age of the host, immunity caused by previous infestations (Parker *et al.*, 1984), and water temperature (Matteson, 1948).

A total of 288 unionids were collected from Moores Ferry (Table 1). Of these, only 10.1% (29 individuals) had their gills modified as marsupia, indicating an 8.93:1 male to female ratio if modified gills are taken to represent females. Marsupial condition is probably not a true representation of the actual male to female ratios, but most unionids are not sexually dimorphic (McMahon, 1991), and the only way to determine the sex of an individual without using histological techniques is to examine the gills for signs of gravidity. However, it would still be expected that at least 50% of the individuals collected would have had

Table 1. Unionids with and without gills modified as marsupia from the Licking River at Moores Ferry, Kentucky.

Taxa	With	Without	Totals
	Modified Gills	Modified Gills	
<i>Actinonaias ligamentina</i> (Lamarck, 1819)	20	135	155
<i>Alasmidonta marginata</i> Say, 1818	0	0	0
<i>Amblesma plicata</i> (Say, 1817)	0	45	45
<i>Cyclonaias tuberculata</i> (Rafinesque, 1820)	0	0	0
<i>Elliptio dilatata</i> (Rafinesque, 1820)	0	18	18
<i>Fusconaia flava</i> (Rafinesque, 1820)	0	2	2
<i>Lampsilis cardium</i> Rafinesque, 1820	2	6	8
<i>Lasmigona complanata</i> (Barnes, 1823)	0	0	0
<i>L. costata</i> (Rafinesque, 1820)	0	6	6
<i>Leptodea fragilis</i> (Rafinesque, 1820)	0	1	1
<i>Megalonaia nervosa</i> (Rafinesque, 1820)	0	9	9
<i>Obliquaria reflexa</i> Rafinesque, 1820	0	0	0
<i>Potamilus alatus</i> (Say, 1817)	1	2	3
<i>Ptychobranchius fasciolaris</i> (Rafinesque, 1820)	6	19	25
<i>Quadrula metanевра</i> (Rafinesque, 1820)	0	0	0
<i>Q. nodulata</i> (Rafinesque, 1820)	0	0	0
<i>Q. pustulosa</i> (Lea, 1831)	0	1	1
<i>Q. quadrula</i> (Rafinesque, 1820)	0	7	7
<i>Tritogonia verrucosa</i> (Rafinesque, 1820)	0	8	8
Totals:	29	259	288

their gills modified as marsupia if normal reproduction were occurring. Histological examination revealed that the male to female ratios for the two target species were not statistically different from 1:1. Both of the target species, as well as other species of unionids, usually maintain a 1:1 male to female ratio (Jirka and Neves, 1992).

Most of the males of the two target species had more than one stage of spermatogenesis present in their gonads, but usually the most advanced stage present dominated the acini of the testes (Table 2). Spermatids were observed in most of the male *Elliptio dilatata* collected, but all the cell types except spermatozoa were also observed. All the cell types except spermatocytes were observed in male *Actinonaias ligamentina*, with sperm morulae and spermatids the most common.

All of the female *Elliptio dilatata* had each of the three egg cell types present in their ovaries (Table 2). The most advanced stage present did not always dominate the

alveoli of the ovaries, as was observed in the testes of the males. Most of the *E. dilatata* were in the second stage of oogenesis with oocytes dominating the alveoli. One female was categorized as unknown because the stage of oogenesis could not be determined due to technical difficulties. Hermaphroditism, which has been reported for both of the target species (van der Schalie, 1970; Jirka and Neves, 1992), was observed in one *Actinonaias ligamentina* individual. Most of the acini in this individual contained male gametes, and those with female gametes seemed to be degenerate. Even though the male to female ratios of the target species were not significantly different from 1:1, a majority of the females had empty marsupia (Table 2).

The post-impoundment water temperatures at Farmers, Kentucky, which is located between Moores Ferry and Cave Run Lake, were found to be significantly lower than pre-impoundment temperatures for the months of May through July. There was no significant change in water temperature for August, and a significant increase for September (Table 3). A change in the temperature regime of an aquatic system, especially a decrease, can have drastic effects on freshwater mussels. Water temperature influences the length of the period of development, the time of fertilization (Matteson, 1948), the release of glochidia (Yokley, 1972; Jirka and Neves, 1992), the survival of glochidia before they attach to a host (Tedla and Fernando, 1969), and it is the major environmental factor that regulates the period of glochidial attachment (Matteson, 1948; Zale and Neves, 1982; Neves and Widlak, 1988). Decreases in water temperature can also result in a decreased production of the crystalline style (Allen, 1921), reduce the motility of adult unionids (Yokley, 1972) and influence the growth of adults (Hruška, 1992).

The average monthly discharge at Farmers, Kentucky, increased from pre- to post-impoundment for the months of June through September and decreased during May, but none of these changes were statistically significant (Table 3). Even though these changes were themselves not statistically significant, they were directly related to significant changes in water temperature. The hydrological variability of a river influences the spatial distribution of individuals in a freshwater mussel community (Di Maio and Corkum, 1995), and stream velocity is a major factor

Table 2. Stages of gametogenesis and marsupial contents of *Actinonaias ligamentina* and *Elliptio dilatata* from the Licking River at Moores Ferry, Kentucky. See text for stage and content descriptions.

Taxa	Stage of Spermatogenesis					Stage of Oogenesis				Marsupial Contents			
	1	2	3	4	5	1	2	3	UNK	EE	AE	MG	EM
<i>Actinonaias ligamentina</i>	1	4	0	4	1	-	-	-	-	2	0	3	12
<i>Elliptio dilatata</i>	1	2	3	7	0	2	4	1	1	0	2	1	5
Totals:	2	6	3	11	1	2	4	1	2	2	2	4	17

Table 3. Mean monthly water temperature and discharge for the Licking River at Farmers, Kentucky, during pre- (1966-1979) and post-impoundment (1991-1994) periods.

Month	Water Temperature (°C)			Discharge (m ³ /s)		
	1966-79	1991-94	Students-t	1966-79	1991-94	Students-t
May	16.3	13.4	3.0291*	48.1	27.7	0.8716
June	22.1	17.0	3.4908*	5.6	14.7	-1.2913
July	24.5	21.3	2.0554*	4.0	8.3	-1.4505
August	23.0	22.9	0.1184	7.6	9.0	-0.3339
September	20.7	23.0	-3.1114*	3.4	5.0	-1.0613

*Statistically different at $\alpha = .05$

determining the presence or absence of different species within a freshwater community (Horne and McIntosh, 1979). During periods of high discharge the dilution of sperm and glochidia may occur (Miller and Payne, 1988), and continuous high-turbulence flow can impact adult unionids by eroding the periostracum (Miller *et al.*, 1984).

Even though there were no changes in discharge, and significant decreases in temperature ($p < 0.05$) only during the months of May through July, the impacts of these changes were still biologically important. It is believed that the average monthly discharge and temperature may not be as important as the spikes of discharge, and the corresponding sudden decreases in temperature, that are caused by the releases of hypolimnion water from Cave

Run Lake during important reproductive periods.

For example, during July 1966, before the completion of Cave Run Lake, water temperature averaged $26.4 \pm 0.85^\circ\text{C}$ and the discharge averaged $3.2 \pm 3.2 \text{ m}^3/\text{s}$ (Fig. 2). During July 1991, after the construction of the lake, the average water temperature was $18.5 \pm 2.82^\circ\text{C}$ and the average discharge was $16.8 \pm 17.5 \text{ m}^3/\text{s}$. There were large spikes of discharge during both of these months. During July 1966 discharge increased and then returned to normal seven days later, resulting in only a 3°C decrease in temperature that did not last long after the increased discharge had subsided. During July 1991 the spike in discharge returned to normal nine days later, and resulted in a 6.5°C decrease in temperature that did not return to its pre-release level until four days later. This single spike of discharge impacted the normal water temperature for a period of 13 days. Sudden, drastic, temperature changes such as these have been reported to cause the abortion of embryos and glochidia (Matteson, 1948; Bruenderman and Neves, 1993).

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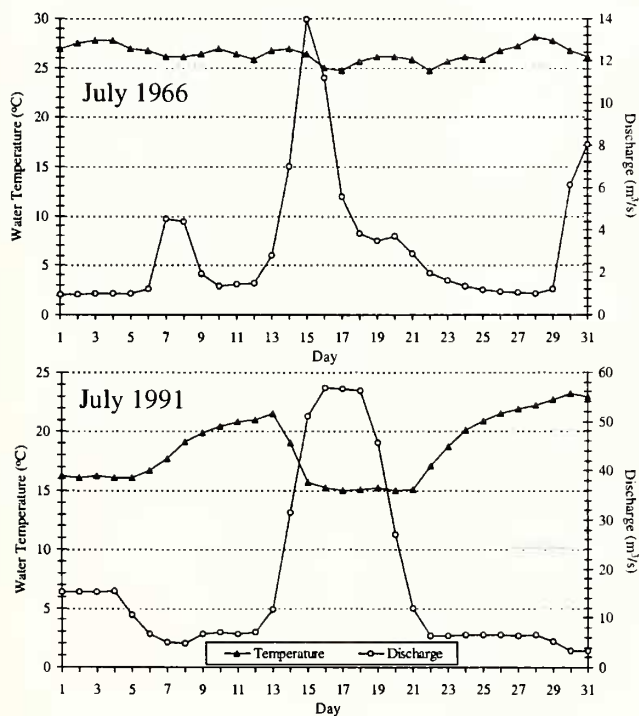


Fig. 2. Licking River at Farmers, Kentucky, mean water temperature ($^\circ\text{C}$) and daily mean discharge (m^3/s) during July 1966 and July 1991.

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